

## Description of *Caenorhabditis auriculariae* n. sp. (Nematoda: Rhabditida) from Fruiting Bodies of *Auricularia polytricha*

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A new *Caenorhabditis* species collected from fruiting bodies of *Auricularia polytricha* is described and illustrated. *C. auriculariae* n. sp. is characterized by the metarhabdion with a bifid flap-like tooth. The caudal papilla 8 bent dorsally and anteriorly bifid brim-like structure on the precloacal lip are also unique to *C. auriculariae* n. sp. These are considered to be autapomorphic characters for this new species. This new species has been obtained also from rotting parts of wood and maintained on bacterial plates of Asparagine-mannitol agar. *Jpn. J. Nematol.* 29(1): 18-23 (1999).

Key words: *Auricularia polytricha*, *Caenorhabditis*, caudal papillae, fruiting body.

The rhabditid genus *Caenorhabditis* (OSCHE, 1952) DOUGHERTY, 1953 is of much interest, since one of its members, *Caenorhabditis elegans* (MAUPAS, 1899) DOUGHERTY, 1953, has become an important model organism for developmental and genetic research. Relatively few studies, however, have focused on the ecology of *Caenorhabditis* (2, 3, 4, 5, 6, 10).

In a survey of nematode fauna on wild mushrooms and toadstools, a new *Caenorhabditis* species was discovered from the fruiting bodies of *Auricularia polytricha* (MONT.) SACC. This species differs in several morphological features from all the known species of *Caenorhabditis*. In this paper we describe the morphology and biology of *C. auriculariae* n. sp.

### MATERIALS AND METHODS

For morphometric and taxonomic studies, *C. auriculariae* n. sp. specimens were obtained from fruiting bodies of *Auricularia polytricha* occurring on trunk of the elder, *Sambucus racemosa* L. subsp. *sieboldiana* (MIQUEL) HARA, in the Botanical Garden of the Faculty of Science in the North Campus, Kyoto University, Kyoto, Japan. The fruiting bodies collected were cut into small pieces by a knife and placed on BAERMANN funnels to extract nematodes. Nematodes obtained were killed in hot water (55°C), fixed in TAF and processed to glycerin.

A sample of decaying parts of the same tree was placed on BAERMANN funnels to obtain nematodes. The nematodes were reared on Asparagine-mannitol agar plate (8).

*Caenorhabditis auriculariae* n. sp.

(Fig. 1)

MEASUREMENTS. See Table 1.

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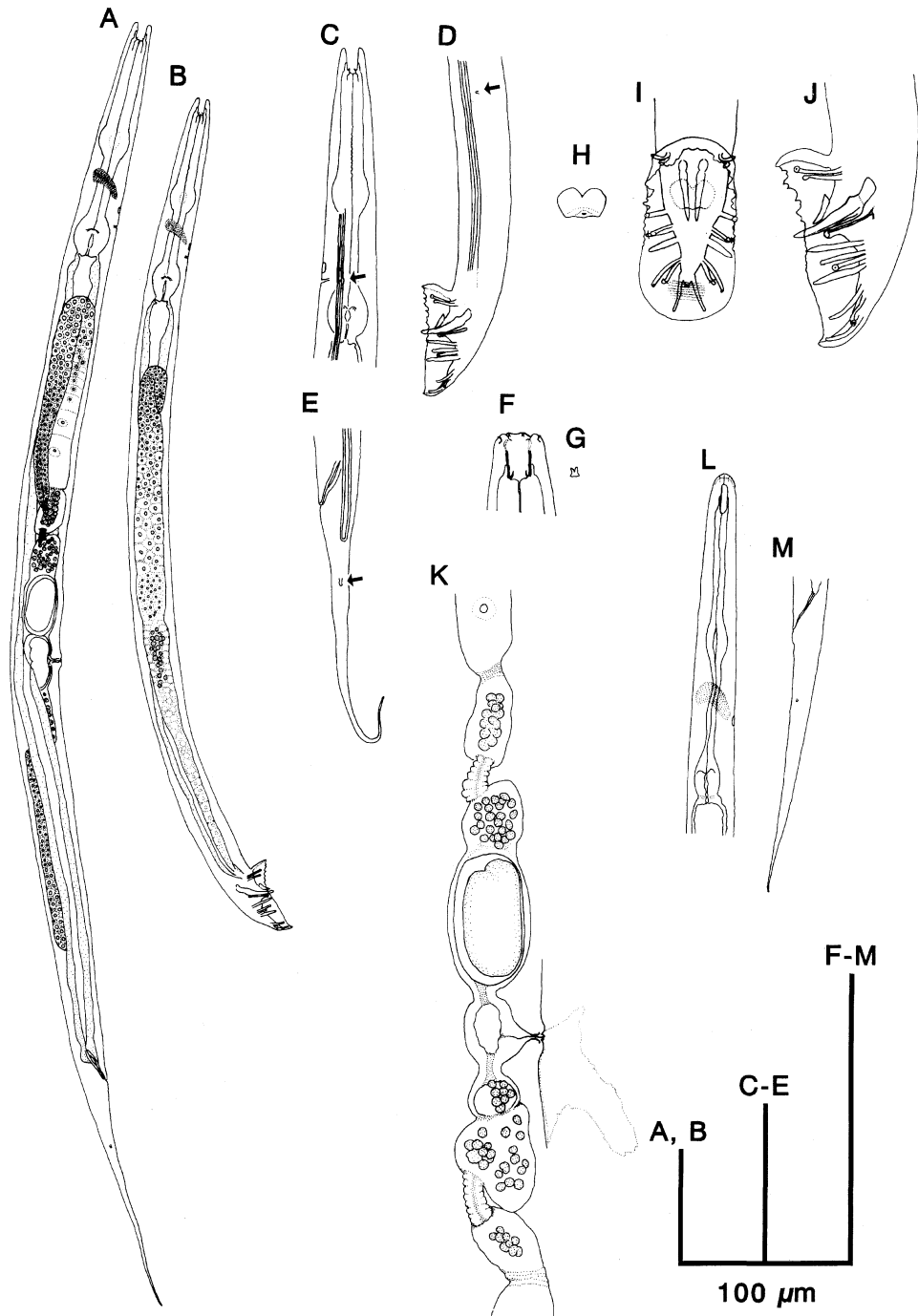


Fig. 1. *Caenorhabditis auriculariae* n. sp. Female. A: right lateral aspect, E: posterior region, lateral (arrow indicates phasmid), F, G: anterior end and metarhabdion, respectively, K: reproductive system. Male. B: right lateral aspect, C: anterior region, lateral (arrow indicates deirid), D: posterior region, lateral (arrow indicates posterior deirid), H: anteriorly bifid cap structure on the precloacal lip, I, J: bursa, ventral and lateral, respectively. Dauer juvenile. L, M: anterior region and posterior region, respectively.

Table 1. Measurements (in  $\mu\text{m}$ ) of *Caenorhabditis auriculariae* n. sp.

Character	Holotype	Females	Males	Dauer juveniles
n	1	15	15	13
L	1115	1069 $\pm$ 98 (930–1250)	783 $\pm$ 69 (650–875)	517 $\pm$ 29 (448–550)
MBW	43.8	43.1 $\pm$ 2.8 (37.5–47.5)	35.0 $\pm$ 2.8 (31.3–40.0)	16.0 $\pm$ 1.7 (13.5–18.5)
Stoma length	14.0	13.8 $\pm$ 0.9 (12.0–15.0)	14.0 $\pm$ 1.0 (12.5–16.0)	9.8 $\pm$ 0.5 (9.0–10.5)
Stoma width	7.0	7.1 $\pm$ 0.3 (6.5–7.5)	6.2 $\pm$ 0.5 (5.3–7.0)	2.0 $\pm$ 0.2 (1.6–2.3)
Pharynx length	200	193 $\pm$ 15 (165–213)	178 $\pm$ 13 (148–198)	119 $\pm$ 5.4 (108–126)
Tail length	195	185 $\pm$ 23 (150–225)	46.8 $\pm$ 3.4 (40.0–50.0)	82.4 $\pm$ 8.7 (56.0–91.0)
ABW	21.8	21.8 $\pm$ 1.8 (18.0–25.0)	27.1 $\pm$ 1.4 (25.0–30.0)	9.5 $\pm$ 0.9 (7.0–10.5)
Hemizonid	153	144 $\pm$ 11 (129–165)	130 $\pm$ 10 (111–146)	86.5 $\pm$ 3.8 (78.8–91.3)
V	49.8	49.8 $\pm$ 1.3 (47.7–52.2)		
Ant. gonad length	480	432 $\pm$ 66 (335–550)		
Post. gonad length	405	368 $\pm$ 48 (295–445)		
Testis length			503 $\pm$ 43 (415–565)	
Spicules			33.7 $\pm$ 2.6 (27.0–38.0)	
Gubernaculum			14.8 $\pm$ 1.0 (13.0–16.0)	
a	25.5	24.8 $\pm$ 1.5 (22.4–27.8)	22.4 $\pm$ 0.9 (20.8–24.7)	32.6 $\pm$ 3.5 (27.8–39.3)
b	5.6	5.5 $\pm$ 0.3 (5.2–5.9)	4.4 $\pm$ 0.2 (3.9–4.7)	4.4 $\pm$ 0.2 (4.0–4.6)
c	5.7	5.8 $\pm$ 0.5 (5.0–7.0)	16.8 $\pm$ 1.1 (15.0–19.2)	6.3 $\pm$ 0.5 (5.8–8.0)
c'	9.0	8.5 $\pm$ 0.7 (6.7–9.3)	1.7 $\pm$ 0.1 (1.6–1.8)	8.7 $\pm$ 0.6 (8.0–10.1)

DESCRIPTION. *Female*. Body straight on relaxation. Cuticle smooth, with fine annules, which are not visible on lateral field. Three lateral ridges extending from the base of median bulb to a point posterior to anus. Head terminates in six lips; each lip with one labial sensillum, but amphids inconspicuous. Stoma relatively short, surrounded by short pharyngeal collar; length of stoma averaging about two times of its width. Metarhabdions isomorphic with a bifid flap-like tooth projecting into the mouth cavity. Pharynx consisting of muscular terminal bulb, narrow isthmus, metacarpus swollen to form median bulb. Nerve ring circles isthmus. Deirids between the center and the dorsal-most ridge of lateral field, at the level of excretory pore which located at or anterior to terminal bulb. Hemizonid anterior to the excretory pore. Vulva a transverse slit, located at midbody. Gonad didelphic and dorsally reflexed, anterior branch right, posterior branch left of the intestine. The reflexed arm of the anterior branch of the gonad represents 55–

79% of the length of the proximal arm (63–94% for the posterior branch). The anterior branch longer than the posterior one. Gonad, measured from anterior to posterior flexure, comprising 40–48 (44)% of body length. Oviduct forming a spermatheca containing sperm cells. Sphincter present between spermatheca and uterus. Oviparous. A few eggs present in uterus. Tail elongate with filiform tip. Phasmid openings 41–56 ( $48 \pm 4$ )  $\mu\text{m}$  behind anus, at 21–32 (25) % of tail length.

*Male.* Body curved ventrally, similar to female in general appearance, usually shorter and thinner. Lateral field extending from the base of median bulb to anterior end of bursa. Testis right of the intestine, comprising 53–64 (60)% of body length; ventrally reflexed part 23–35 ( $31 \pm 3$ )  $\mu\text{m}$  long, that is 5–8 (6)% of gonad length. Seminal vesicle and vas deferens ventral to the intestine. Male tail peloderan, bursa ellipsoid and anteriorly closed, posterior tip typically rounded. Edge of the bursa waved largely anterior to caudal papilla 3, edge waved slightly or smooth between papillae 3 and 6, edge usually smooth posterior to papilla 6. Phasmids at distal tip of tail. Caudal papillae 2+1+3+3 pattern, papilla 6 slightly thicker than others. Caudal papilla 8 bented dorsally. Distal tips of caudal papillae 1, 5 and 8 attached to dorsal surface of bursa, 2, 4, 7 and 9 to ventral surface and 3 to edge. Tips of caudal papillae 6 embedded in velum without reaching the edge. Gubernaculum almost straight, half as long as spicule. Distal end of gubernaculum turned dorsally. Spicules robust with dorsal velum, slightly curved ventrally, unfused, distal tips pointed. Anteriorly bifid cap structure on the precloacal lip. Posterior deirids visible in some specimens, 155–223  $\mu\text{m}$  anterior to tail tip, at about 75% of body length.

*Dauer juveniles.* Mouth closed. Median and terminal bulb weakly developed, diameter of median bulb 6.7–8.5  $\mu\text{m}$ , of terminal bulb 7.8–9.5  $\mu\text{m}$ . Deirids conspicuous, situated 86–98  $\mu\text{m}$  behind the anterior end. Lateral field with five lateral ridges. Tail conical with filiform tip.

TYPE HABITAT AND LOCALITY. Fruiting bodies of *Auricularia polytricha* (MONT.) SACC. generated on the trunk of *Sambucus racemosa* L. subsp. *sieboldiana* (MIQUEL) HARA in the Botanical Garden of the Faculty of Science in the North Campus, Kyoto University, Kyoto, Japan.

TYPE SPECIMENS. Holotype (slide No. 9901) and paratypes (slide No. 9902–9931) deposited in the Nematode Collection at the Laboratory of Environmental Mycospore Science, Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

DIAGNOSIS AND RELATIONSHIPS. *C. auriculariae* n. sp. is characterized by a bifid flap-like tooth on each metarhabdion in both sexes, and ellipsoidal bursa anteriorly closed with waved margin in the anterior half of the velum, papillae pattern (2+1+3+3), dorsally bented papilla 8, and precloacal lip with anteriorly bifid brim structure in males.

Most *Caenorhabditis* species possess one single triangular tooth (2, 4, 7, 9), though in *C. plicata* the armature of the metarhabdions consists of three ridges each (5, 7). *C. auriculariae* n. sp. differs from all these known *Caenorhabditis* species in possessing a bifid flap-like tooth on each metarhabdion. The waved (serrated) margin in the anterior half of the velum and the papillae pattern (2+1+3+3) of *C. auriculariae* n. sp. are shared by *C. elegans*, *C. formosana*, *C. remanei*, *C. oncomelaniae* in “*Elegans*-group” (7). *C. auriculariae* n. sp., however, differs from “*Elegans*-group” in the ventral shape of bursa; heart-shaped and terminally notched in “*Elegans*-group” whereas ellipsoid and terminally rounded in *C. auriculariae* n. sp. The broad, anteriorly bifid brim of the structure on the precloacal lip is unique to *C. auriculariae* n. sp. and not known in other *Caenorhabditis* species including “*Elegans*-group”. These differences suggest that *C.*

*auriculariae* n. sp. does not belong to “*Elegans*-group”. The papillae 7 in all *Caenorhabditis* species described are bent dorsally and open to the dorsal side of the velum (7). In *C. auriculariae* n. sp., however, the orientation of papilla 7 and 8 is reversed.

BIOLOGY AND ECOLOGY. *C. auriculariae* n. sp. was found in association with fruiting bodies of *A. polytricha*. It is unknown whether this association is specific or not. But it is not a requisite part of the *C. auriculariae* n. sp. life cycle, because they have been obtained from rotting part of wood and maintained on bacterial plates.

*C. auriculariae* n. sp. is gonochoristic and oviparous. Males are as abundant as females. Females carry less than four eggs in their uterus, but never juveniles. Fertilized embryos reach adulthood in about five days on bacterial plates at 20°C.

Male moves back and keeps contact its bursa with female body to search vulva during precopulation behavior. They copulate in a parallel manner. During copulation, they make a  $\lambda$ -form configuration. They do not remain stationary, large female draws small male usually. Copulation can last for several minutes. Male deposits a gelatinous mating plug at the vulva of female.

When food is scarce, dauer juveniles will be formed. Dauer juveniles are motile when prodded otherwise remain stationary.

## DISCUSSION

*C. auriculariae* n. sp. can be distinguished from other *Caenorhabditis* species based on the structure of metarhabdion, the arrangement of caudal papillae, and the structure of precloacal lip. On the basis of the cladogram and the data compiled for other *Caenorhabditis* species (7), the papilla 8 bent dorsally and anteriorly bifid brim-like structure of precloacal lip are considered to be autapomorphic characters for *C. auriculariae* n. sp.

*C. auriculariae* n. sp. was obtained both from fruiting bodies of *A. polytricha* and from decaying wood. From fruiting bodies, a great number of *C. auriculariae* n. sp. (about 1,400 individuals from 6 fruiting bodies) were detected solely, whereas only three individuals of *C. auriculariae* n. sp. were detected with many other species of free living nematodes from decaying wood. This fact suggests that *C. auriculariae* n. sp. may have a particular way to invade and colonize the fruiting body of *A. polytricha*. One possible way is to be vectored by some invertebrates. Several associations between *Caenorhabditis* species and other invertebrates, e.g. snails, slugs, cadaver beetles, pill bugs and *Drosophila* flies have been described (2, 4, 5, 10). Therefore *C. auriculariae* n. sp. may have phoretic or necromenic associations with some of such invertebrates in its life cycle.

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#### 和文摘要

### アラゲキクラゲ *Auricularia polytricha* の子実体から検出された *Caenorhabditis auriculariae* n. sp. の記載

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アラゲキクラゲ *Auricularia polytricha* の子実体から検出された *Caenorhabditis* 属の 1 新種を *Caenorhabditis auriculariae* n. sp. として記載した。この種は、二又状の歯を備えた後部口腔壁によって特徴づけられる。また、8 番目の交接嚢腺が交接翼の外側に向かって開口していることや総排出腔開口部の前唇の形状はこの種独特の派生形質であると考えられた。この種は、アラゲキクラゲ子実体だけでなくその発生木の腐朽部からも検出され、アスパラギン・マンニトール培地上で細菌を餌として維持することが可能であった。